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Oviposition preference of the cottonwood leaf beetle, Chrysomela scripta F., on poplar clones, Populus spp.

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OVIPOSITION PREFERENCE OF THE COTTONWOOD LEAF BEETLE, CHRYSOMELA SCRIPTA F., ON POPLAR CLONES, POPULUS SPP.

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Oviposition preference of the cottonwood leaf beetle, *Chrysomela scripta* F., on poplar clones, *Populus* spp.

by

Dennis Allen Haugen

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INTRODUCTION

In recent years, intensive culture of poplars, Populus spp., has received much attention and research effort in the United States and Canada (USDA-Forest Service 1976, 1983; Dickman and Stuart 1983). Hybrid poplar clones are being developed for use in intensively managed forest systems to produce fiber, fuel, and sawlogs. This short-rotation crop system is a monoculture with trees of uniform age, size, spacing, and species with limited genetic diversity (one or a few clones). These modifications in the biological and physical environment disrupt the complex interactions among the organisms within a natural stand, and usually favor an increase in insect and pathogen populations. Thus, intensive culture poplar plantations are generally more susceptible to pest outbreaks than are natural stands (Wilson 1976, 1977; McNabb et al. 1982). Over 150 species of insect pests are known to attack poplars in the eastern United States (Baker 1972). Populus also is affected by many diseases including leaf spots, leaf rusts, and stem cankers.

The cottonwood leaf beetle (CLB), Chrysomela scripta Fabricius, is an important defoliator of Populus throughout the eastern United States. Adults and larvae feed on foliage and meristematic tissue, sometimes destroying apical shoots. Damage is most severe in nurseries and in plantations of

young trees. Severe infestations can result in substantial growth loss, reduction in tree form and wood quality, and mortality of young trees. More information on the biology and ecology of the CLB than is currently available is needed to construct an effective pest management system. This system also must be compatible with the overall intensive culture management system as well as with other pest management systems for pathogens and insects on Populus. Inclusion of host plant resistance of poplars to CLB in this system may be a feasible method to reduce the damage caused by this insect.

A few studies have investigated adult feeding preferences (Harrell et al. 1981), larval development times (Harrell 1980), and field injury by adults and larvae on selected poplar clones (Caldbeck et al. 1978; Wilson 1979). Another important area to be investigated in the cottonwood leaf beetle-hybrid poplar interaction is oviposition preference. Oviposition preference determines the larval food, since the larval foraging range is limited to the tree on which eggs are oviposited. Larvae are considered to be the most damaging stage, because they are confined to one tree and are present in greater numbers than adults (Harrell et al. 1982). If a clone is not preferred for oviposition, damage to that clone would be reduced because of the reduced number or lack of CLB larvae.

Objectives of this study were to determine oviposition

preferences of the cottonwood leaf beetle on poplar clones from the three major sections of Populus (Leuce, Aigeiros, and Tacamahaca), and to compare oviposition preferences to clonal rankings for adult feeding preferences (Harrell et al. 1981), larval development times (Harrell 1980), and field injury by adults and larvae (Caldbeck et al. 1978; Wilson 1979).

LITERATURE REVIEW

Poplars and Intensive Culture

Poplars are prime candidates for use in intensive culture forest plantation systems in North America and around the world (Dickmann and Stuart 1983; FAO 1979). Demand for wood products (timber, pulp, and fuelwood) is expected to increase as the forest land base continues to decrease (Dickmann and Stuart 1983). In an intensive culture system, trees are grown in a short rotation (1 to 20 years) under nearly optimized conditions, e.g., spacing, nutrients, water, weed control, and protection from pests. The establishment and management of an intensive culture forest plantation (Hansen et al. 1983) have many aspects which resemble agricultural crop systems more than traditional forest management practices. The short rotation time, thus return on the investment, allows for the input of intensive management practices which are not feasible under normal forest management. Currently, intensive culture is not economically feasible in most situations in the United States, but economic assessments on intensive culture for future application have been positive, with some reservation until more information is gathered (Bentley 1975; Rose and Kallstrom 1976; Rose and DeBell 1978).

The genus Populus has three major sections and three

minor sections (Dickmann and Stuart 1983). Major sections are Leuce (aspens and white poplars), Aigeiros (cottonwoods and black poplars), and Tacamahaca (balsam poplars). Minor sections are Turanga (one species), Leucoides (four species), and Abaso (one species), none of which are of great economic significance. Many species of poplars can be crossed with one another, but generally crosses within a section are more easily obtained than crosses between sections (Knox et al. 1972). An exception to this is crosses between the Aigeiros and Tacamahaca sections, which are easy to obtain (Schreiner 1971; Smith 1943). Crosses between Leuce and Aigeiros or Leuce and Tacamahaca poplars are typically difficult and often produce empty seeds or low vigor seedlings (Dickmann and Stuart 1983).

The first comprehensive North American poplar breeding project was conducted by Stout and Schreiner (1933) for the Oxford Paper Company of Maine in cooperation with the New York Botanical Garden. The hybrid crosses from this project have been widely distributed in North America, and many of the Northeastern and North Central Forest Experiment Stations Populus clones are from this early project (Dickmann and Stuart 1983).

Cottonwood Leaf Beetle

The cottonwood leaf beetle (CLB) occurs throughout the United States and Canada and feeds on leaves of poplar,

willow, and alder (Baker 1972). Adult beetles overwinter under loose bark or debris. Adults emerge in early spring and feed on new leaves and succulent shoots. Eggs are deposited on the undersides of leaves in masses of 15 to 75 (Baker 1972). The beetle has three larval instars (Burkot and Benjamin 1979). Early instar larvae are gregarious and skeletonize (windowpane) leaves. Later instar larvae become less gregarious and consume all layers of leaf tissue, leaving just the larger veins. Pupation occurs on leaves, bark, weeds, or grass. Adults emerge and begin feeding and mating. Oviposition occurs five to six days after adult emergence (Head and Neel 1973). Females lay six to ten egg masses with a total egg production of 350 to 650 eggs/female (Burkot and Benjamin 1979). Four generations/year have been reported in Wisconsin (Burkot and Benjamin 1979) and seven generations/year in Mississippi (Head et al. 1977).

Insecticides have been screened and application procedures have been proposed for control of CLB (Morris 1960; Neel 1969; Page and Lyon 1976; Abrahamson et al. 1977). Insecticides can provide adequate protection from CLB, but large scale applications should be minimized due to the potential environmental hazard (Dickmann and Stuart 1983). When possible, systemic insecticides should be used rather than broad-spectrum contact insecticides to decrease the impact on nontarget insects and other organisms. Other methods of CLB management also should be considered (e.g.,

silvicultural control, sanitation, biological control, and host plant resistance).

Few parasites and predators of CLB have been reported (Neel et al. 1976; Head et al. 1977; Burkot and Benjamin 1979). Head et al. (1977) reported a ladybird beetle, Coleomegilla maculata DeGeer, as the most common predator. Two parasites, Schizonatus latus (Walker) (Hymenoptera: Pteromalidae) and an unidentified tachinid fly also were reported, but were less abundant than C. maculata. Brown (1956) explained that the lack of parasites and predators was due to protective glands on CLB larvae. All larval instars of the CLB possess nine pairs of eversible glands (two thoracic and seven abdominal). These glands expose droplets of salicylaldehyde, which provide larvae with a repellent shield (Wallace and Blum 1969). Salicylaldehyde is conserved by retracting the droplet into the gland shortly after exposure, and is retained during larval molts.

Insect-Plant Interactions

Complex interactions between phytophagous insects and their host plants are the result of long evolutionary processes of each species counteracting the other's strategies for survival. The field of host plant resistance is involved intimately in these interactions. The goal of host plant resistance is to reduce insect damage by "tipping the scale" in favor of the host plant. Painter (1951)

assembled the first comprehensive work of insect resistance in crop plants. Early work by Fraenkel (1959, 1969) discovered the relationship between secondary plant substances and host selection of insects. Subsequently, many significant papers concerning host plant resistance have been published (Painter 1958; Thorsteinson 1960; Beck 1965; Schoonhoven 1968; Levin 1976; Dethier 1982).

Host plant resistance to insects has been divided into three categories: nonpreference, antibiosis, and tolerance (Painter 1951). Nonpreference, later termed antixenosis (Kogan and Ortman 1978), is comprised of plant characteristics and insect behaviors which repel or do not attract insects to the plant. Antibiosis is any plant mechanism which has an adverse effect on growth and survival of the insect. Tolerance is the plant's ability to withstand an infestation which would severely damage susceptible plants.

Biochemical and morphological characteristics of host plants have been related to an insect's behavior in host selection (Kennedy 1965; Norris and Kogan 1980; Dethier 1982; Miller and Strickler 1984). These plant characteristics are the foundation of understanding insect-plant interactions and mechanisms of host plant resistance. These characteristics also can be used to explain the ecological host range of an insect, i.e., monophagous, oligophagous, or polyphagous (Jermy 1961).

Much of host plant resistance research has dealt with larval and adult feeding behaviors. Oviposition behavior is important with insect species in which immatures are not mobile or acceptable host plants are widely scattered. Host selection behaviors for oviposition of a few insects have been studied, e.g., a chrysomelid beetle (Phillips 1976, 1977), a sphingid moth (Yamamoto et al. 1969), a swallowtail butterfly (Wiklund 1974), a pierid butterfly (Behan and Schoonhoven 1978), and a nymphaline butterfly (Singer 1982).

The use of host plant resistance in forest pest management is becoming more feasible. Breeding trees for resistance to insects has been slower than in agricultural crops because of long generation intervals, few forest geneticists, use of natural regeneration instead of planting improved stock, and lack of knowledge about tree physiology, insect biology, and their interactions (Hanover 1975, 1980). Examples of research on developing and applying tree resistance to insects and pathogens of forest trees can be found in Gerhold et al. (1966) and Heybroek et al. (1982).

Cottonwood Leaf Beetle-Poplar Interactions

Cottonwood leaf beetles are the most serious defoliators of intensive culture poplars. Host plant resistance is a promising method of CLB management in intensive culture poplar plantations (Harrell 1980). Field observations of poplar clones for CLB injury suggested some degree of

resistance among Populus species and their hybrids (Caldbeck et al. 1978; Wilson 1979). A wide variation in CLB resistance was found in eastern cottonwood along the southern Mississippi River (Oliveria and Cooper 1977). Adult feeding preference has been quantified for a few hybrid poplar clones and these results extrapolated to predict adult feeding preference for various Populus sectional crosses (Harrell et al. 1981). Larval development time on various Populus clones has been investigated (Harrell 1980).

Other recent studies have added to the knowledge of CLB ecology and interaction with poplars (Burkot and Benjamin 1979; Harrell et al. 1982; Head et al. 1977). Susceptibility of willow clones to CLB also has been investigated (Randall 1971). Similar host plant resistance research has been conducted in Europe with leaf beetles (Coleoptera: Chrysomelidae), e.g., Phyllodecta vitellinae L. on poplars (Finet and Gregoire 1981, 1982; Finet et al. 1983) and Galerucella lineola L. on willows (Wiren and Larsson 1983).

MATERIALS AND METHODS

Oviposition preference of the cottonwood leaf beetle (CLB) was investigated in greenhouse experiments and in a field plot. Greenhouse cages were used in multiple choice and no choice experiments using 12 poplar clones. A field plot was planted with the same 12 clones. Oviposition preference was quantified by the mean number of egg masses and the mean number of eggs/mass for each clone.

Clone Selection

Poplar clones for this study were selected from the North Central Forest Experiment Station (NCFES) Populus clones used by Harrell (1980). These clones represented a broad range of susceptibilities to cottonwood leaf beetle damage as determined by Caldbeck et al. (1978) and Wilson (1979) (Table 1) and were from the list of leading candidates for use in the North Central region at the time of these studies. Ten clones were selected from the 12 clones in Harrell's study, so comparisons could be made between adult feeding preference and oviposition preference. Two clones eliminated from Harrell's selection were NCFES clones 5266 and 5331; however, clones with the same Populus species as parents were in the ten selected clones in this study (NCFES clones 5334 and 5332, respectively). Two additional clones were selected, a

native eastern cottonwood, Populus deltoides (IS-31) and a native bigtooth aspen, Populus grandidentata, which represented poplars native to Iowa. Parentage and sectional composition of the 12 selected clones are shown in Table 2. Statistical comparisons of the means from these sectional composition groups were made as an extrapolation or prediction of ovipostion preference for clones with the same sectional composition. These comparisons only considered one to four clones for each group, thus the selected clones may not be representative of a sectional group.

Clonal materials to propagate 11 of these clones were available from a poplar clonal orchard maintained by the Department of Forestry, Iowa State University. Roots of a bigtooth aspen clone were collected from a natural stand at Holst State Forest, near Boone, Iowa. Bigtooth aspen cuttings were obtained from root suckers which sprouted from these roots. Stock plants for the other poplar clones were started from hardwood cuttings obtained from the clonal orchard at Wilcox Experimental Farm.

Tree Propagation

Poplar trees were propagated as softwood cuttings from stock plants (Faltonson 1983; Faltonson et al. 1983). Cuttings were placed in a rooting bed with overhead intermittent mist in the greenhouse. The more difficult to root clones, 5339, 5377, and native P. deltoides, were

treated with a rooting hormone (IBA, 500 ppm, 5 sec dip of rooting end of cutting) before being placed in the mist bed. After the cuttings developed root systems (approximately 2 weeks), rooted cuttings were planted in plastic pots (10 cm in diameter) with a peat-vermiculite potting medium. Potted cuttings were placed in a semi-shaded location in the greenhouse to prevent wilting of the very succulent tissue. After a week, the trees were moved to greenhouse benches and allowed to grow in full sunlight. Height growth was rapid and trees suitable for the greenhouse experiments were obtained in another 2 weeks. Therefore, a total of 5 to 6 weeks was needed for propagation of experimental trees from softwood cuttings.

Cottonwood Leaf Beetle Laboratory Colony

A laboratory colony of CLB was needed due to the near absence of a natural field population in central Iowa in the spring and summer of 1984. The extremely low CLB population was hypothesized to be the result of heavy mortality of overwintering adults and first generation larvae caused by a combination of severe early winter temperatures, little snow cover, and heavy spring and early summer rains. An observation of a mating pair of CLB in the field plot was made 27 June 1984, but no eggs or adults were found in subsequent observations.

A collection of CLB was made at the University of

Minnesota Experimental Farm, near Rosemount, Minnesota, on 23 July 1984. All life stages were collected from a mixed clonal hybrid poplar plantation. Cottonwood leaf beetles were placed in plastic crispers (30 x 20 x 10 cm) and transported in ice coolers (ca. 10°C) to Iowa State University. Life stages were sorted, placed in respective crispers, and held in an environmental growth chamber (Percival^{R} Model I-35-VL) at 24°C (16 hr) and 18°C (8 hr) with a photoperiod (light:dark) regime of 16:8 hr to simulate summer conditions in Iowa.

Beetles were fed succulent leaves from a native P. deltoides clone (IS-31). They were fed daily and transferred to clean crispers every 2 to 4 days. Adult beetles were transferred to clean crispers (20 to 30 adults per crisper) after emerging. These crispers served as mating chambers and oviposition occurred on leaves and crisper sidewalls. At higher population densities (> 100 beetles/crisper) egg production was reduced. Eggs from the mating chambers were used to start the next generation of the laboratory colony. Laboratory generations were noted and recorded. The multiple choice greenhouse experiment used adults from the original (Rosemount, MN) field collection, and adults for the no choice experiment were from the first generation laboratory colony.

Field Plot

Oviposition preference of the CLB was investigated in a mixed clonal Populus field plot. The plot (0.275 ha) was located at the Iowa State University Hinds Experimental Farm, ca. 1 km north of Ames, Iowa. This site was selected because of its sandy-loam soil and availability of an irrigation system. Also, poplars had been grown on the site and natural CLB infestations had occurred in previous years.

Poplar trees, ca. 10 years old, were cleared and stumps removed from the site in April and May 1983. The plot was disked and roto-tilled before planting. The area to be planted (52 x 22 m) was divided into 6 blocks, each block with 12 subplots. Within each block, the 12 clones were randomly assigned to the 12 subplots. A subplot, the experimental unit, consisted of 12 trees of the same clone planted at a 1 x 1 m spacing in a 4 x 3 tree arrangement. Therefore, the plot contained 864 potential sample trees, 72 trees of each clone. Two border rows, 280 trees, surrounded the entire plot with the 12 clones assigned randomly in clonal groups of three or four trees.

Trees for the field plot were propagated using softwood cuttings in June 1983. After two weeks under intermittent mist, the rooted cuttings were potted. The trees grew in the greenhouse for one week, then were placed outside to harden off before planting. The plot was planted on 6 July 1983.

Planted trees were between 0.2 and 0.6 m tall at the time of planting. The plot was irrigated with an overhead irrigation system the day of planting, then at weekly intervals for 5 weeks. Each irrigation was for 4 to 5 hours. Weed control in the plot was initially by hoeing the entire plot manually, then later by mowing between the rows in two directions and hoeing around each tree.

The sampling scheme was to select four trees randomly within each subplot and record for each tree the height, number of leaves, number of CLB egg masses, number of eggs in each mass, relative location of the egg mass on leaves within the tree canopy (e.g., top, mid, or lower third, and main stem or lateral branch), presence of other CLB life stages, and presence of other insects. The mean of these four subsamples was the experimental unit of the analysis. After CLB naturally infested the plot, data were taken at 10 to 14 day intervals.

Greenhouse Experiments

Oviposition preference also was investigated in the controlled environment of greenhouse cages. The cages were located in a separate bay of the insectary greenhouses on the Iowa State University campus. Cages were made of nylon mesh with an internal 0.9 x 1.1 x 0.9 m wood frame.

Poplar trees for the greenhouse experiments were propagated as described previously. Mean heights of the

trees used in the experiments were ca. 0.5 m. The source of CLB adults for the greenhouse experiments was a laboratory colony.

Multiple choice

In the multiple choice experiment, one tree of each clone was assigned a position randomly inside a screen cage, for a total of 12 trees per cage. Each tree was considered as an experimental unit. Adult CLB (unsexed) were selected randomly from mating chambers of the laboratory colony. Five to eight adult beetles were released from a petri dish placed in the center of the cage, and they were allowed to feed and oviposit. After 3 to 4 days, adults were counted and removed from the cages. Trees were searched for egg masses. For each egg mass, data were recorded for clone, tree position, tree height, number of leaves on the tree, relative egg mass location within the tree, number of eggs, number of other egg masses on that tree, and presence or absence of adult feeding on that tree. Eight or nine cages were used for each time period and this was repeated over six time periods for 50 replications. These tests were completed between 24 July and 13 August 1984.

No choice

In the no choice experiment, six trees of the same clone were placed inside each screen cage. Adult beetles were

released in the cages and, after 3 to 5 days, data were recorded as described for the multiple choice tests. Ten cages were used for each time period and this was repeated over six time periods for five replications for each clone. These tests were completed between 13 August and 4 September 1984. Clones were assigned randomly to cages, so a clone would not be tested more than once for a time period and no clone would be assigned to a given cage more than once over the six time periods. The experimental unit was the cage, so cage means were calculated and used in the analysis.

RESULTS

Field Plot

Cottonwood leaf beetle (CLB) adults were first sighted in the field plot in mid-August 1983. By late August, a small natural infestation had started. Field plot samples were taken on 26 August, 8 September, and 23 September 1983. Four trees in each subplot were sampled, for a total of 288 sample trees for each sampling period. The total number of egg masses found on the sampled trees for the three sampling periods were 6, 10, and 11 egg masses repectively. Field plot sampling was discontinued after the third sample due to a termination of oviposition activity, most probably caused by an early frost on 23 September. A summary of egg mass frequencies by clone and date for 1983 is shown in Table 3. Statistical analyses of these data for clonal differences were not warranted because of the low frequency of CLB egg masses.

Of the 27 egg masses sampled, the mean egg count was 55.6 eggs/mass (s=11.9) with a range of 31 to 75 eggs (Fig. 1). Most egg masses, 70.4%, were found on leaves attached to the main stem in the upper third of the canopy, while the remaining egg masses, 29.6%, were found on leaves attached to the main stem or lateral branches in the lower two-thirds of the canopy.

For 1984, the field plot was monitored for the occurrence of an active CLB infestation. No CLB adults or eggs were found until 27 June 1984, when a mating pair was observed in the field plot. No CLB life stages were found in subsequent observations made during the next two weeks.

An attempt to infest the field plot artificially with CLB collected from Rosemount, Minnesota was unsuccessful. Adult CLB were released five times in the plot (a total of 800 adults released) during a 3 week period (26 July to 17 August). Adults, egg masses, and first instar larvae were observed during this period, but in very low numbers. Data were collected on 23 August 1984, but no egg masses were found on the sampled trees.

Greenhouse Experiments

Multiple Choice

In the multiple choice experiment, 50 cage replications were completed with 12 trees per cage for a total of 600 trees (tree = experimental unit). A total of 235 egg masses were oviposited on the 600 experimental trees. A summary of egg mass frequency by clone is shown in Table 4.

The LOGIT model was used to analyze these data for clonal differences. Analysis of variance (ANOVA) was not appropriate due to the great frequency of observations with zero for a value. The LOGIT model used the following

equation:

$$\hat{P}_{ij} = \frac{e^{(\hat{\mu} + \hat{R}_i + \hat{C}_j)}}{1 + e^{(\hat{\mu} + \hat{R}_i + \hat{C}_j)}}$$

where $\hat{P}_{ij}$ = probability of a tree having at least one egg mass for each REP and CLONE, $\hat{\mu}$ = estimated mean effect over all REP and CLONE, $\hat{R}_i$ = estimated REP effect for each period (i=6), $\hat{C}_j$ = estimated CLONE effect for each clone (j=12).

Data were fit to the model by the FUNCAT procedure of SAS with the maximum likelihood option and suppression of the generalized least-squares estimates (SAS Institute Inc. 1982). The FUNCAT model predicted the probability of egg masses for each REP and CLONE, then compared the predicted value with the observed value by the chi-square test. The INTERCEPT, REP, and CLONE parameters of the model were found to be significantly different from zero using the chi-square test (Table 5). The LIKELIHOOD RATIO, a test for lack of fit, was not significant, which suggested the model had a good fit to the data. Contrasts between sectional groupings showed a highly significant difference ($p<0.001$) in probabilities of an egg mass between Aigeiros clones and Leuce clones, between the Tacamahaca clone and Leuce clones, and between Aigeiros clones and 25% Aigeiros + 75% Tacamahaca clones (Table 5). Significant differences ($p<0.05$) were

found between Aigeiros clones and 50% Aigeiros + 50% Tacamahaca clones and between 50% Aigeiros + 50% Tacamahaca clones and 25% Aigeiros + 75% Tacamahaca clones. No difference was found between Aigeiros and Tacamahaca groups ($p<0.9151$).

The mean number of eggs in the 235 egg masses was 57.5 ($s=15.4$) with a range of 5 to 82 eggs, and the median was 61.5 eggs per mass. A frequency distribution of egg counts showed a peak in the 65-69 interval (Fig. 2). Contrasts of mean number of eggs per mass were made between sectional groupings and no significant differences were found between sectional groupings (Table 6). Sectional means ranged from 60.3 eggs/mass for 50% Aigeiros + 50% Tacamahaca clones to 46.4 eggs/mass for Leuce clones.

Locations of egg masses on the trees were recorded and summarized. Egg masses on expanding leaves of the terminal shoot (99/235 masses) and the rest of the upper third of the tree canopy (103/235 masses) accounted for 86.0% of the egg masses oviposited on the experimental trees. Leaves from the middle third of the tree had 13.2% of the egg masses oviposited on them, while less than 1% of the egg masses were found on leaves from the lower third of the canopy.

Multiple egg masses were found on the same tree and same leaf in many observations. Egg masses ($n=235$) were found on 148 of 600 experimental trees. Single egg masses occurred on 97 trees, two masses occurred on 30 trees, three masses

occurred on 11 trees, four masses on seven trees, five masses on two trees, and seven masses occurred on one tree. Multiple egg masses on the same leaf occurred 20 times with two egg masses in 18 occurrences and three egg masses on the same leaf with two occurrences. Only five egg masses were found on the upper surface of the leaves; therefore, 97.9% of the egg masses were found on the lower surface of the leaves. The mean egg count in these five masses was 32.2 eggs, which was substantially less than the overall mean of 57.5 eggs/mass. Twenty-one egg masses were laid on other surfaces in the cages beside leaf tissue. Of these egg masses, 66.7% were laid on the cage screening and the other masses were found on tree pots and petri dishes.

In every observation, a tree with an egg mass had some adult leaf feeding. Adult feeding was frequently on the leaf with an egg mass. This suggests that the adult feeds on a tree before oviposition. This evidence is not conclusive, since feeding and oviposition were not observed directly. In a few cases, the leaf tissue was almost completely consumed around an egg mass; therefore, some of the adult feeding occurred after oviposition.

No Choice

In the no choice greenhouse experiment, 60 cage observations (6 trees/cage = experimental unit) were completed with five replications for each of the 12 clones.

A total of 102 egg masses were oviposited during the 60 cage observations (360 trees). A summary of the egg mass frequency by clone is presented in Table 7.

The valid application of tests of significance in the analysis of variance (ANOVA) requires that experimental errors be distributed independently and normally with a common variance (Steel and Torrie 1980). Data that consist of small whole numbers, e.g., count data, does not meet these basic assumptions for the ANOVA. Data must be transformed to meet the assumptions and for a valid test of significance to be conducted. For the type of data collected in this experiment (egg mass counts with some zero values), the most appropriate transformation was:

$$TMASS = SQRT(MASS + 0.5)$$

where MASS = egg mass count for the six trees/cage, and TMASS = transformed MASS data.

An ANOVA for egg mass frequency (Table 8) showed a highly significant difference ($F=3.87$, $p<0.0007$) in oviposition preference among clones. Contrasts between sectional groupings revealed a highly significant difference between Aigeiros clones (1.95 mass/cage) and Leuce clones (0.30 mass/cage) ($p<0.0002$), and between the Tacamahaca clone (2.20 mass/cage) and Leuce clones ($p<0.0017$). The difference between 50% Aigeiros + 50% Tacamahaca clones (2.47 mass/cage) and 25% Aigeiros + 75% Tacamahaca clones (1.20 mass/cage) was almost statistically significant ($p<0.0567$).

The mean number of eggs in the 102 egg masses was 58.5 (s=12.4) with a range of 13 to 77 eggs and a median of 60 eggs. A frequency distribution of the egg count showed a peak in the 60-65 interval (Fig. 3). Contrasts of mean number of eggs per mass were made between sectional groupings and no significant differences were found between any of the groupings (Table 9). Sectional means ranged from 61.7 eggs per mass for Leuce clones to 56.5 eggs per mass for 25% Aigeiros + 75% Tacamahaca clones.

Locations of egg masses on the trees were recorded and summarized. Egg masses on the expanding leaves of the terminal shoot (38/102 masses) and the rest of the upper third of the tree canopy (52/102) accounted for 88.2% of the egg masses oviposited on the experimental trees. Leaves from the middle third of the tree had 7.8% of the egg masses oviposited on them, while less than 4% of the egg masses were found on leaves from the lower third of the canopy. Egg masses were found in 40 of the 60 cage observations. Egg masses (n=102) were found on 77 of 360 experimental trees. Single egg masses occurred on 57 trees, two masses occurred on 16 trees, three masses occurred on three trees, and four masses occurred on one tree. Multiple egg masses on the same leaf were found seven times and, in each occurrence, two masses were found. In every occurrence, a tree with an egg mass had some adult leaf feeding. Only one egg mass (51 eggs) was found on the upper surface of a leaf. Thirty-four

egg masses were oviposited on other surfaces beside leaf tissue. Of these masses, 64.7% were laid on the cage screening and 32.4 % were laid on tree pots. No significant differences were found between sectional groupings in the number of egg masses oviposited on these non-leaf surfaces. Mean number of eggs for these non-leaf surfaces was 32.4 eggs/mass which is less than the mean of 58.5 eggs/mass found on leaf surfaces in this no choice experiment.

DISCUSSION

Field Plot

Low field populations of the cottonwood leaf beetle (CLB) in 1983 resulted in few egg masses in the field plot. Only 27 egg masses were found on sample trees, which was a very small proportion of egg masses to sample trees (27/864 = 0.03). No statistical analysis was done on these data, but a trend toward CLB oviposition preference of Aigeiros clones over clones from the other sections was suggested from the limited data.

Unsuccessful attempts to infest the field plot artificially in 1984 were hypothesized to be due to the same causes of extremely low field populations that year. A mid-summer drought and hot temperatures caused the trees to stop growth, thus no succulent tissue was available for beetle feeding. Egg masses found in the field plot hatched, but first instar larval mortality was great. Evidence of first-instar larval feeding was observed, but the feeding area was small in comparison to normal feeding, and very few larvae were found.

Greenhouse Experiments

Multiple choice

The multiple choice experiment was used to determine CLB

oviposition preference for a Populus clone relative to the other Populus clones present. Cottonwood leaf beetle females were given 12 Populus clones from which to select oviposition sites, which would simulate a multi-clonal plantation.

Aigeiros and Tacamahaca clones were highly preferred for CLB ovipositon in the multiple choice experiment, while sectional hybrids were moderately preferred. Two clones from the Leuce section were avoided for oviposition, with only 8 egg masses oviposited on 100 trees from this section (Table 4). Antixenosis (non-preference) of Leuce clones by CLB was expected since aspens are not natural hosts of the CLB (Baker 1972).

Comparison of the Aigeiros and Tacamahaca clones showed no significant difference in oviposition preference, but only 1 clone of the Tacamahaca section was used in the tests. Native hosts of the CLB include species from both Aigeiros and Tacamahaca sections, but Aigeiros species are more common over the range of the CLB and especially in the midwestern United States. Comparisons of the Aigeiros group with the 50% Aigeiros + 50% Tacamahaca and 25% Aigeiros + 75% Tacamahaca groups showed a relationship in which the greater the percentage of Aigeiros parentage the greater the oviposition preference. The relationship did not include the 100% Tacamahaca clone (NCFES 5260), which was highly preferred (Table 5). The inconsistent relationship of oviposition preference might be due to having only one

Tacamahaca clone, which was not representative of the Tacamahaca section for oviposition preference, or might be due to hybrid vigor of sectional crosses in which the hybrids were less preferred than either Populus section of the parents. Possible mechanisms of this resistance in the hybrids include synergistic chemicals, changes in morphological characteristics, or a combination of these factors.

No significant differences were found among clones or sectional groupings for mean number of eggs/mass (Table 6). Leuce clones had the lowest mean egg count, 46.4 eggs/mass, but due to the low number of masses on Leuce clones, the difference was not significant when compared with the other section means. From the multiple choice experiment results, CLB oviposition behavior relative to host suitability was described by the number of egg masses oviposited on a clone. The number of eggs/mass was independent of host suitability.

No choice

The no choice experiment was used to determine CLB oviposition preference for a Populus clone when other clones were not available. The response was an "accept" or "reject" choice. Female CLB could oviposit or not oviposit on trees of a single clone. This test gave information on CLB behavior and clonal preference in a "mini-monoculture" system.

Aigeiros and Tacamahaca clones were preferred for oviposition when compared to Leuce clones (Table 8). Aspens and white poplars of the Leuce section are not natural hosts of the CLB. Only three egg masses were oviposited on the two Leuce clones (60 trees) in the no choice experiment. The mechanism for resistance of Leuce clones was antixenosis (non-preference), which operates before oviposition, most likely during the adult examining phase (tactile, gustatory, visual, and/or olfactory stimuli).

No significant differences for mean number of eggs/mass were found between clones or sectional groupings (Table 9). Leuce clones had the highest mean egg count, but also had the lowest frequency of egg masses. Egg counts of the three masses on Leuce clones were 57, 57, and 71 eggs. So only one egg mass was responsible for the high mean.

Comparison of greenhouse experiments

Results from the multiple choice and no choice greenhouse experiments showed similar relationships of sectional groupings (Tables 5 and 8). In both experiments the Leuce section had the lowest egg mass frequency, while the Aigeiros and Tacamahaca sections were not significantly different in egg mass frequency. The relationship between sectional hybrids for egg mass frequency were similar, with 50% Aigeiros + 50% Tacamahaca clones more preferred than 25% Aigeiros + 75% Tacamahaca clones. This relationship was

significant in the multiple choice experiment ($p<0.0350$), and it was almost significant in the no choice experiment ($p<0.0567$). An inconsistency occurred between the results of the greenhouse experiments for egg mass frequency of Aigeiros and 50% Aigeiros + 50% Tacamahaca groups. In the multiple choice experiment, egg mass frequency was significantly greater in the Aigeiros group compared to the 50% Aigeiros + 50% Tacamahaca group. In the no choice experiment, the 50% Aigeiros + 50% Tacamahaca group had a greater egg mass frequency than the Aigeiros group, but the difference was not significant. The difference in results between the greenhouse experiments was investigated by a comparison of individual clones.

A comparison of egg mass frequencies of the individual clones from the multiple choice and no choice greenhouse experiments was done by rank order (Table 10). The order of Populus clones was consistent between greenhouse experiments except for the order reversal of clones 5322 and 5334. Clone 5322 was the most preferred clone in the multiple choice experiment, but one of the least preferred clones in the no choice experiment. Clone 5334 had the opposite relationship; one of the least preferred clones in the multiple choice experiment and the most preferred clone in the no choice experiment.

A biological reason for this reversal in CLB clonal preference is not readily apparent. The change in relative

position of clone 5334 may have a plausible explanation. Other clones have greater stimuli for oviposition relative to clone 5334 in the multiple choice test. While in the no choice test, stimuli for oviposition between the clones are of the same magnitude and oviposition occurs at equal frequencies since the beetles have only one choice, oviposit or not.

The other change in relative position, clone 5322, does not have an apparent biological explanation. It is not feasible that a clone would be selected over the other clones in a multiple choice test, and then be relatively "non-preferred" when only trees of that clone were offered.

An obvious hypothesis to explain the reversal of these two clones is the mislabeling of trees in one of the greenhouse experiments. This is plausible, since the trees for the multiple choice experiment were propagated at one time and three weeks later the no choice experiment trees were propagated. A mislabeling could have occurred when the cuttings were taken from the stock plants, when the cuttings were placed in the mist bed, or when the rooted cuttings were potted.

An experiment will be done this summer (1985) to observe if these results are repeatable or if the hypothesis of mislabeling is supported. No choice tests will be conducted with clone 5334 and 5322. Multiple choice tests also will be done with these clones and other clones from the original

experiment included as base-line standards.

Comparison of Field and Greenhouse Experiments

Mean egg mass frequency of the sectional groups from the field plot and greenhouse experiments were compared (Table 11). Mean egg mass frequency values from these three experiments are from different experimental designs and represent subplot, individual tree, and cage means. Therefore, the means are used only for comparison of relative position within an experiment and are not directly comparable between experiments. Leuce egg mass frequencies were consistently the lowest when compared to the other sectional groups, while clones from the Aigeiros section ranked high in egg mass frequency in the three experiments. The 25% Aigeiros + 75% Tacamahaca group was the second least preferred group for oviposition. The 50% Aigeiros + 50% Tacamahaca group was highly variable in its ranking of CLB oviposition preference between experiments. It ranked first in the no choice experiment, was moderately preferred in the multiple choice experiment, and was in the cluster of four non-preferred groups in the field plot. A plausible explanation for some of this variability is the question raised about possible mislabeling of an Aigeiros clone and a 50% Aigeiros + 50% Tacamahaca clone in the no choice greenhouse experiment. Another explanation of this variability would be differences in the physiological state

of the clones during the various experiments, thus affecting the acceptability of those clones for CLB oviposition.

The Tacamahaca section showed a great change in rank from being highly preferred in both greenhouse experiments to no oviposition on this clone in the field plot. The low preference of the Tacamahaca clone (5260) in the field plot may be due to a random non-event in a small sample of egg masses, or due to the clone's early cessation of growth compared to the other clones. Field plot data were collected in late August and September, at which time clone 5260 lacked succulent leaf tissue. Early and midseason CLB oviposition was hypothesized to occur on this clone. Trees, including clone 5260, used in the greenhouse experiments were growing vigorously and had succulent leaf tissue.

Mean egg counts/mass were very similar between the three experiments; the field plot averaged 55.6 eggs/mass, the multiple choice experiment averaged 57.5 eggs/mass, and the no choice experiment averaged 58.5 eggs/mass (Figs. 1, 2, 3). Standard deviations for the mean egg counts were ca. 12-15 eggs, and the range from 5 to 82 eggs/mass. In a laboratory study, Burkot and Benjamin (1979) observed a mean of 64.3 eggs/mass (s=14.7 eggs) with a range of 5 to 148 eggs/mass for 10 females which oviposited 81 egg masses.

No differences were found among clones for egg mass count in the greenhouse experiments. Due to the low number of egg masses in the field plot, comparison of egg counts among

clones in the field plot was not done. From these results, CLB oviposition behavior seems to be a threshold response to the stimuli from a tree. If stimuli pass the threshold level, an egg mass will be oviposited, but if stimuli do not pass the threshold level, no oviposition will occur. Variability in the number of eggs per mass may be due to age of female, number of egg masses previously oviposited, and other physiological factors of the female CLB. An oviposition behavior of varying the number of eggs oviposited relative to clonal preference is not supported by these data.

Relative location of CLB egg masses within the tree canopy showed the highest frequency on leaves from the top third of young trees (<1 year) including the expanding leaves from the terminal shoot. In the three experiments, 70-90% of the egg masses were found in the top third of the canopy. Oviposition on mid-canopy leaves was much less frequent, 5-25% of the masses. Lower leaves had the lowest occurrence of egg masses, less than 5% of the egg masses. High egg mass frequency on leaves in the top third of the tree may be due to the physiological state of the leaves, e.g., succulent new leaf tissue, or visual orientation of the female for the top of small trees, or a combination of these factors. Burkot (1978) observed CLB egg masses about half way up one year old trees. He stated that CLB life stages tended to segregate vertically into three layers: 1) a top layer with second and third instar larvae and feeding adults, 2) a middle layer

with ovipositing adults, eggs, and first instar larvae, and 3) a bottom layer with prepupae, pupae, and teneral adults. The difference in egg mass location between these two observations may be due to CLB population levels. In this oviposition study, the greenhouse trees had no previous feeding on leaves, and the field injury due to adult and larval feeding was minimal. In contrast, a field observation at moderate to high CLB populations would have trees with previous larval and adult feeding on the upper leaves and oviposition may be displaced to mid-canopy leaves.

Comparison with Other Studies

Results from this oviposition preference study were compared with results from studies on CLB adult feeding preference (Harrell et al. 1981), CLB larval development time (Harrell 1980), and CLB adult and larval feeding injury (Caldbeck et al. 1978; Wilson 1979). The purpose of these comparisons was to note trends and differences in CLB preferences for Populus clones for different activities. Data from the studies were not directly comparable, since different parameters were measured and different experimental designs were used. Results from the multiple choice oviposition preference greenhouse test were used in the comparison since the other studies were multiple choice tests.

Adult feeding preference

The relative ranking and comparison between CLB adult feeding and oviposition preferences are shown in Table 12. Rankings between studies were fairly consistent, considering different experimental conditions. The Tacamahaca clone (5260) was highly preferred in both studies. Leuce clones ranked at the bottom (least preferred) in both studies. Generally, clones of 50% Aigeiros + 50% Tacamahaca composition were moderately preferred by CLB for oviposition and adult feeding.

The most obvious difference in clonal rankings was the 25% Aigeiros + 75% Tacamahaca clones (5262 and 5263), which were highly preferred for CLB adult feeding but ranked very low for CLB oviposition. Studies which ranked Populus clones based on CLB adult and larval injury (Caldbeck et al. 1978; Wilson 1979) showed relatively great injury to clone 5263 (Table 1). Clone 5262 had the greater injury relative to the other clones in Wilson's (1979) study, but had a moderate level of injury in the Caldbeck et al. (1978) study. Another large difference in ranking between the studies was found with clone 5264. This clone was not preferred in the adult feeding test, but was highly preferred in the oviposition test (Table 12). From the adult and larval injury studies (Table 1), clone 5264 was the most preferred clone in one study (Caldbeck et al. 1978), but moderately preferred in the other study (Wilson 1979).

Populus clones varied in their ranking of CLB preference between the adult feeding and ovipositon studies. Some clones were preferred or rejected in both studies, but other clones were preferred in one study and rejected in the other study. Differences in clonal preferences between the studies may be due to differences in the physiological states of the trees. The reversal in preferences between studies also may be due to different mechanisms of host selection for oviposition versus adult feeding. Adult CLB host selection for oviposition and feeding may be the result of two independent gene complexes, in which different host plant factors are stimuli for host plant selection of each adult activity (i.e., feeding or oviposition). Different host selection mechanisms for oviposition and adult feeding may reduce competition between adult and larval stages of the CLB for a food source.

Other factors also may be involved in the differences in results between oviposition and adult feeding preferences. These studies had many unaccounted variables between them, which may cause these differences in clonal ranking. Possible factors include CLB population density, source of CLB adults (e.g., genetic differences; wild population vs. laboratory colony), age of trees, time of year (physiological differences among Populus clones), and other unaccounted beetle and tree variables.

Larval development time

The relative ranking and comparison of Populus clones between larval development time and oviposition preference are shown in Table 13. Clonal rankings were consistent between the studies, e.g., clones with relatively high oviposition preference had relatively fast larval development times. These results supported a hypothesis that oviposition occurs on host plants which allow for optimum larval development.

Field injury by larvae and adults

Relative ranking and comparison of Populus clones among the field injury studies (Caldbeck et al. 1978; Wilson 1979) and oviposition preference study are shown in Table 14. Results between the two field studies showed a fairly consistent ranking among the ten clones with the exception of clones 5262 and 5264. A comparison of the 33 clones in both studies (Table 1) showed a general consistency in clonal ranking considering differences in data collection and environmental conditions. A few clones (e.g., 5273, 5262, 5328, and 5264) have contrasting relative positions in the comparison between these two studies.

Clonal ranking between CLB oviposition preference and field injury observations shows greater differences in rankings (Table 14). In one difference in ranking, Clone 5322 was highly preferred for oviposition, but had a moderate

to low level of injury in the field studies. However, a clone with a high oviposition preference may rank at a low to moderate level of injury in the field due to other biological factors. In this case, clone 5322 was moderately preferred for adult feeding (Harrell et al. 1981). Also, the suitability of the clone for larval development will influence the amount of injury. No estimate of larval suitability for this clone is available, since it was not included in the larval development study (Harrell 1980). In other differences in ranking, clones 5262, 5263, and 5334 were not highly preferred for oviposition, but ranked high in CLB injury in the field studies. These high levels of injury in the field may be due to adult feeding, which is supported by high adult feeding preferences of these clones found by Harrell et al. (1981).

CONCLUSIONS

Populus clones in the Aigeiros and Tacamahaca sections used in this study were more preferred for oviposition than Leuce clones. Hybrid clones between Aigeiros and Tacamahaca sections from this study were generally less preferred for CLB oviposition than either of the parent sections, especially hybrids of 25% Aigeiros + 75% Tacamahaca composition. Oviposition preference was determined by the number of egg masses laid on each clone. No difference was found in the mean number of eggs/mass among the clones. Oviposition behavior of CLB was an accept/reject response to the stimuli from a tree, in contrast to varying the number of eggs/mass relative to clonal preference. Location of egg deposition within a tree showed a great frequency on leaves from the top third of young trees (<1 year), including expanding leaves from the terminal shoot.

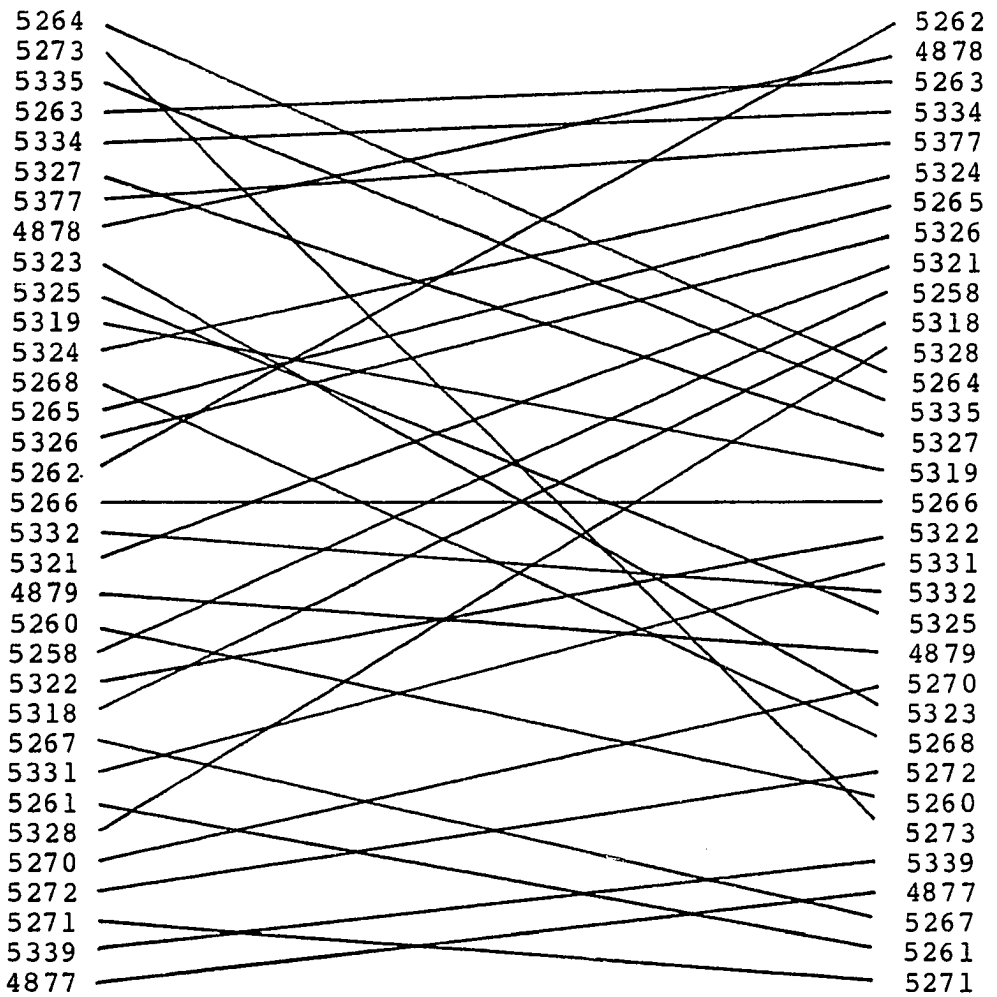
Comparisons of Populus clones for relative rank of oviposition preference and other CLB activities showed some inconsistencies in clonal rank for oviposition and adult feeding preferences. These results suggest different mechanisms of host selection for oviposition versus adult feeding. However, a comparison of oviposition preference with larval development times revealed a very consistent relationship between the two activities. Level of field

injury to Populus clones by the CLB can be explained, at least partially, by a combination of adult feeding preference, larval development time, and oviposition preference.

Comparisons among these studies were done by rankings, since data were not directly comparable among studies. Further research would be advised which combined adult feeding and oviposition preferences, so that these data could be analyzed statistically and compared to corroborate or contradict these initial conclusions. Other CLB behavior studies are needed to understand the host selection process for oviposition and adult feeding, and to understand the cottonwood leaf beetle-hybrid poplar interaction in intensive culture systems.

This study has shown differences among Populus clones in CLB oviposition preference. Clones which rank low in oviposition preference, adult feeding preference, and field injury level are candidates for use in an intensive culture poplar plantation, if these clones also meet other criteria, e.g., growth characteristics, clone suitability to site, and disease resistance. Existing and newly developed hybrid crosses between the Aigeiros and Tacamahaca sections should be screened for CLB resistance. Hybrid poplars with Leuce and either Aigeiros or Tacamahaca composition also should be screened for CLB resistance once poplar breeders are able to mass produce these hybrids.

Table 1. Comparison of cottonwood leaf beetle injury between Iowa and Minnesota Populus study plots (modified from Wilson 1979)



[a] North Central Forest Experiment Station (NCFES) Populus clone number, ranked from greatest to least injury.

Table 2. *Populus* clones used in this cottonwood leaf beetle oviposition preference study

NCFES[a] clone #	Parentage	Sectional[b] Composition
5339	*P. alba* x *P. grandidentata*	L
-	*P. grandidentata* (native)	L
5322	*P.* x *euramericana*	A
5377	*P.* x *euramericana*	A
5264	*P. deltoides*[c] x *P. nigra*[d]	A
-	*P. deltoides* (native), "IS-31"	A
5332	*P. nigra*[e] x *P. trichocarpa*	HA
5334	*P. deltoides*[c] x *P. trichocarpa*	HA
5272	*P. nigra* x *P. laurifolia*	HA
5262	*P. balsamifera*[f] x (*P.* x *berolinensis*)[g]	QA
5263	*P. balsamifera*[f] x (*P.* x *berolinensis*)[g]	QA
5260	*P. tristis* x *P. balsamifera*	T

[a] NCFES *Populus* clone numbers.

[b] A = 100% *Aigeiros*,
T = 100% *Tacamahaca*,
L = 100% *Leuce*,
HA = 50% *Aigeiros* + 50% *Tacamahaca*,
QA = 25% *Aigeiros* + 75% *Tacamahaca*.

[c] *P. deltoides* var *angulata*.

[d] *P. nigra* var *plantierensis*.

[e] *P. nigra* var *betulifolia*.

[f] *P. balsamifera* var *subcordata* = *P. candicans*.

[g] *P.* x *berolinensis* = *P. nigra* var *italica* x *P. laurifolia*.

Table 3. Cottonwood leaf beetle egg mass count in *Populus* field plot in 1983

Clone[a]	Egg Mass Count			
	26 Aug.	8 Sept.	23 Sept.	Total
5339	0	0	0	0
P. grandidentata	0	0	0	0
5322	1	2	0	3
5377	3	2	4	9
5264	0	0	1	1
P. deltoides	2	5	2	9
5332	0	0	0	0
5334	0	1	1	2
5272	0	0	1	1
5262	0	0	0	0
5263	0	0	2	2
5260	0	0	0	0
Total	6	10	11	27

[a] NCFES *Populus* clone number.

Table 4. Cottonwood leaf beetle egg mass frequency by *Populus* clone in multiple choice greenhouse experiment

Sectional[a] Composition	Clone[b]	Mean Egg Mass[c] Frequency	Sectional Mean
L	5339	0.04	0.08
L	*P. grandidentata*	0.12	
A	5322	0.72	0.58
A	5377	0.50	
A	5264	0.72	
A	*P. deltoides*	0.36	
HA	5332	0.46	0.37
HA	5334	0.28	
HA	5272	0.36	
QA	5262	0.16	0.22
QA	5263	0.28	
T	5260	0.70	0.70

[a] A = 100% *Aigeiros*,
T = 100% *Tacamahaca*,
L = 100% *Leuce*,
HA = 50% *Aigeiros* + 50% *Tacamahaca*,
QA = 25% *Aigeiros* + 75% *Tacamahaca*.

[b] NCFES *Populus* clone numbers.

[c] 50 observations/clone.

Table 5. LOGIT model analysis and contrasts of egg mass probability in multiple choice greenhouse experiment

Source	df	Chi-Square	PROB
INTERCEPT	1	111.72	0.0001
REP	5	46.74	0.0001
CLONE	11	40.75	0.0001
LIKELIHOOD RATIO	55	57.21	0.3932

Contrasts[a]	PROB of an Egg Mass	Chi-Square	PROB
A vs T	0.36 vs 0.36	0.01	0.9151
A vs HA	0.36 vs 0.26	3.91	0.0480
A vs QA	0.36 vs 0.15	13.94	0.0002
HA vs QA	0.26 vs 0.15	4.44	0.0350
L vs A	0.05 vs 0.36	21.09	0.0001
L vs T	0.05 vs 0.36	18.04	0.0001

[a] Sectional composition,
A = 100% Aigeiros,
T = 100% Tacamahaca,
L = 100% Leuce,
HA = 50% Aigeiros + 50% Tacamahaca,
QA = 25% Aigeiros + 75% Tacamahaca.

Table 6. Contrasts of mean egg counts/mass by *Populus* sectional composition, and mean egg count by clone in multiple choice greenhouse experiment

Contrast[a]	Mean Egg Count	F-Value	PR > F
A vs T	46.4 vs 56.8	3.00	0.0846
A vs HA	56.8 vs 56.6	0.09	0.7609
A vs QA	56.8 vs 59.4	0.40	0.5295
HA vs QA	60.3 vs 59.4	0.00	0.9493
L vs A	46.4 vs 56.8	3.00	0.0846
L vs T	46.4 vs 56.6	2.35	0.1267

Sectional[a] Composition	Clone[b]	N	Mean Egg Count
A	5377	25	61.4
HA	5272	18	60.8
A	delt.	18	60.8
HA	5334	14	60.6
QA	5262	8	60.0
HA	5332	23	59.8
QA	5263	14	59.0
T	5260	35	56.6
A	5264	36	55.7
A	5322	36	52.6
L	5339	2	52.5
L	grand.	6	44.3

[a] A = 100% *Aigeiros*,
T = 100% *Tacamahaca*,
L = 100% *Leuce*,
HA = 50% *Aigeiros* + 50% *Tacamahaca*,
QA = 25% *Aigeiros* + 75% *Tacamahaca*.

[b] NCFES *Populus* clone number,
delt. = *P. deltoides*,
grand. = *P. grandidentata*.

Table 7. Cottonwood leaf beetle egg mass frequency by *Populus* clone in no choice greenhouse experiment

Sectional[a] Composition	Clone[b]	Mean Egg Mass[c] Frequency	Sectional Mean
L	5339	0.0	0.3
L	*P. grandidentata*	0.6	
A	5322	1.2	2.0
A	5377	2.2	
A	5263	3.2	
A	*P. deltoides*	1.2	
HA	5332	2.2	2.5
HA	5334	3.6	
HA	5272	1.6	
QA	5262	1.4	1.2
QA	5263	1.0	
T	5260	2.2	2.2

[a] A = 100% *Aigeiros*,
T = 100% *Tacamahaca*,
L = 100% *Leuce*,
HA = 50% *Aigeiros* + 50% *Tacamahaca*,
QA = 25% *Aigeiros* + 75% *Tacamahaca*.

[b] NCFES *Populus* clone number.

[c] Mean of 5 cage observations (6 trees/cage).

Table 8. Analysis of variance and contrasts of egg mass count in no choice greenhouse experiment

Source	df	Mean Square	F-Value	PR > F
MODEL	16	0.7433	4.53	0.0001
CLONE	11		3.87	0.0007
REP	5		5.99	0.0003
ERROR	43	0.1641		
TOTAL	59			

Contrasts[a]	Mean Egg Mass Frequency	F-Value	PR > F
A vs T	1.95 vs 2.20	0.34	0.5617
A vs HA	1.95 vs 2.47	0.52	0.4746
A vs QA	1.95 vs 1.20	2.04	0.1605
HA vs QA	2.47 vs 1.20	3.84	0.0567
L vs A	0.30 vs 1.95	16.02	0.0002
L vs T	0.30 vs 2.20	11.18	0.0017

[a] Sectional composition,
A = 100% Aigeiros,
T = 100% Tacamahaca,
L = 100% Leuce,
HA = 50% Aigeiros + 50% Tacamahaca,
QA = 25% Aigeiros + 75% Tacamahaca.

Table 9. Contrasts of mean egg counts/mass by *Populus* sectional composition, and mean egg count by clone in no choice greenhouse experiment

Contrast[a]	Mean Egg Count	F-Value	PR > F
A vs T	57.4 vs 60.8	0.05	0.8334
A vs HA	57.4 vs 59.3	1.79	0.2009
A vs QA	57.4 vs 56.5	1.48	0.2426
HA vs QA	59.3 vs 56.5	0.00	0.9962
L vs A	61.7 vs 57.4	0.24	0.6293
L vs T	61.7 vs 60.8	0.37	0.5530

Sectional[a] Composition	Clone[b]	N	Mean Egg Count
L	grand.	3	61.7
T	5260	11	60.8
HA	5272	8	60.5
HA	5334	18	59.4
HA	5332	11	58.2
A	delt.	6	58.0
A	5322	6	57.8
A	5377	11	57.7
A	5264	16	56.8
QA	5262	7	56.8
QA	5263	5	56.4
L	5339	0	-

[a] A = 100% *Aigeiros*,
T = 100% *Tacamahaca*,
L = 100% *Leuce*,
HA = 50% *Aigeiros* + 50% *Tacamahaca*,
QA = 25% *Aigeiros* + 75% *Tacamahaca*.

[b] NCFES *Populus* clone number,
grand. = *P. grandidentata*,
delt. = *P. deltoides*.

Table 10. Comparison of cottonwood leaf beetle oviposition preference by clonal ranking in multiple choice and no choice greenhouse experiments

MULTIPLE CHOICE		NO CHOICE	
Mean[a] Egg Mass Frequency	Clone[b]	Clone	Mean[c] Egg Mass Frequency
0.72	5322	5334	3.6
0.72	5260	5264	3.2
0.70	5264	5260	2.2
0.50	5377	5377	2.2
0.46	5332	5332	2.2
0.36	5272	5272	1.6
0.36	delt.[d]	5262	1.4
0.28	5334	delt.	1.2
0.28	5263	5322	1.2
0.16	5262	5263	1.0
0.12	grand.[e]	grand.	0.6
0.04	5339	5339	0.0

[a] Individual tree mean.

[b] NCFES *Populus* clone number.

[c] Cage mean (6 trees/cage).

[d] *P. deltoides*.

[e] *P. grandidentata*.

Table 11. Comparison of cottonwood leaf beetle egg mass frequency by sectional grouping for field plot and greenhouse experiments

FIELD PLOT		MULTIPLE CHOICE		NO CHOICE	
Section[a] Comp.	Mean[b] Egg Mass Frequency	Section Comp.	Mean[c] Egg Mass Frequency	Section Comp.	Mean[d] Egg Mass Frequency
A	0.31	T	0.70	HA	2.47
HA	0.06	A	0.58	T	2.20
QA	0.06	HA	0.37	A	1.95
T	0.00	QA	0.22	QA	1.20
L	0.00	L	0.08	L	0.30

[a] Sectional composition of clones within group
A = 100% Aigeiros,
T = 100% Tacamahaca,
L = 100% Leuce,
HA = 50% Aigeiros + 50% Tacamahaca,
QA = 25% Aigeiros + 75% Tacamahaca.

[b] Subplot mean (4 sample trees).

[c] Individual tree mean.

[d] Cage mean (6 trees/cage).

Table 12. Comparison of Populus clonal rankings for cottonwood leaf beetle oviposition and adult feeding preferences (Harrell 1980)

OVIPOSITION PREFERENCE		ADULT FEEDING PREFERENCE	
Mean[a] Value	Clone[b]	Clone	Mean[c] Value
0.72	5322	5260	836
0.72	5264	5262	664
0.70	5260	5263	632
0.50	5377	5377	600
0.46	5332	5331	580
0.36	5272	5322	512
0.36	delt.[d]	5334	376
0.28	5334	5266	348
0.28	5263	5332	344
0.16	5262	5272	316
0.12	grand.[e]	5264	208
0.04	5339	5339	8

[a] Mean egg mass frequency in multiple choice greenhouse experiment.

[b] NCFES Populus clone number.

[c] Mean leaf area consumed (mm^2).

[d] P. deltoides.

[e] P. grandidentata.

Table 13. Comparison of Populus clonal rankings for cottonwood leaf beetle oviposition preference and larval development times (Harrell 1980)

OVIPOSITION PREFERENCE		LARVAL DEVELOPMENT	
Mean[a] Value	Clone[b]	Clone	Mean[c] Value
0.72	5264	5377	13.9
0.70	5260	5332	13.9
0.50	5377	5264	14.1
0.46	5332	5260	14.3
0.36	5272	5272	14.3
0.28	5334	5263	14.4
0.28	5263	5334	14.5
0.16	5262	5262	14.6
0.04	5339	5339	none[d]

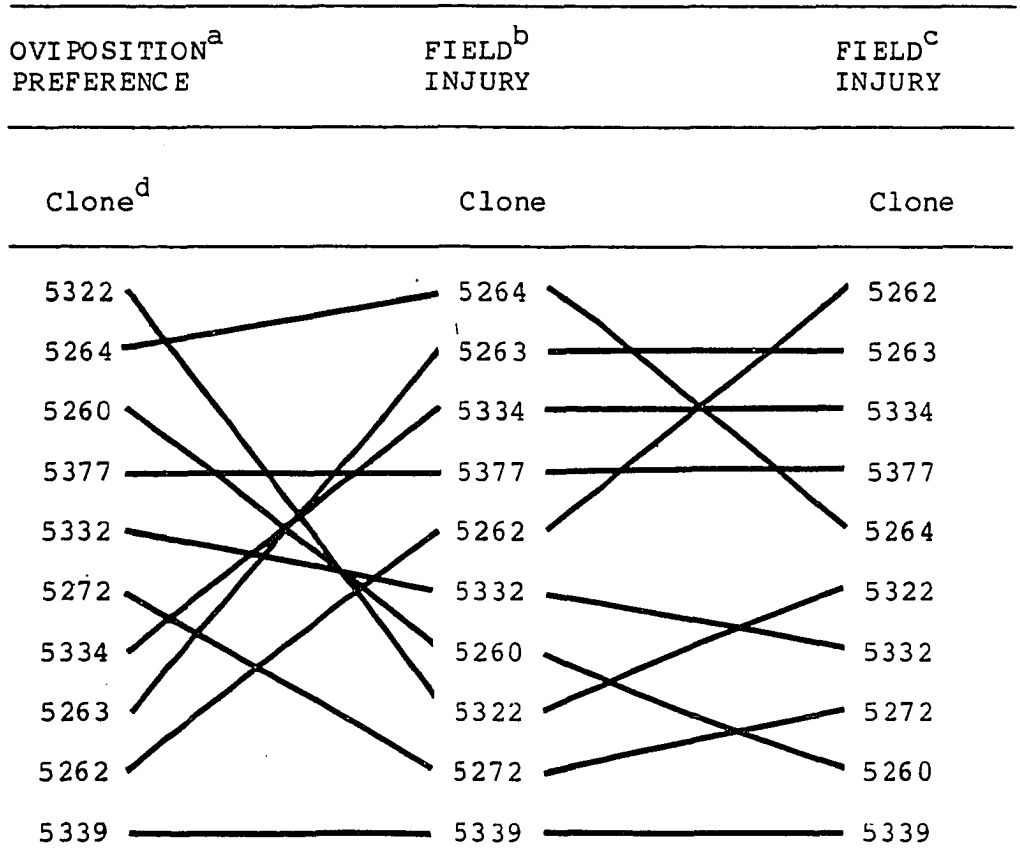
[a] Mean egg mass frequency in multiple choice greenhouse experiment.

[b] NCFES Populus clone number.

[c] Mean larval development (days).

[d] Larvae did not survive.

Table 14. Comparison of Populus clonal ranking for cottonwood leaf beetle oviposition preference and field injury by larvae and adults (Caldbeck et al. 1978; Wilson 1979)



[a] Multiple choice greenhouse experiment.

[b] Caldbeck et al. 1978, Iowa study.

[c] Wilson 1979, Minnesota study.

[d] NCFES Populus clone number, ranked from greatest to least injury/oviposition.

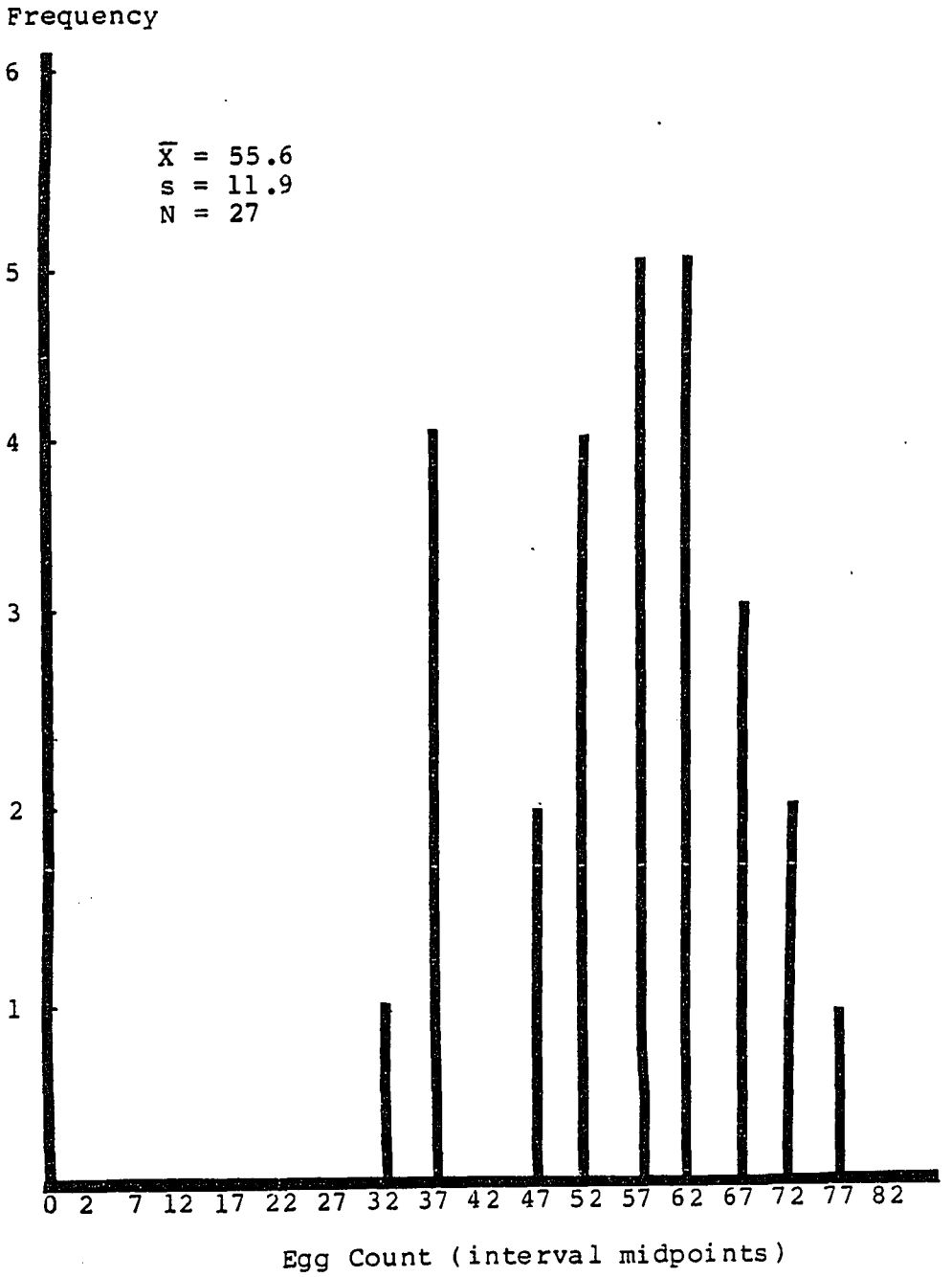


Fig. 1. Frequency of egg counts/mass in field plot experiment

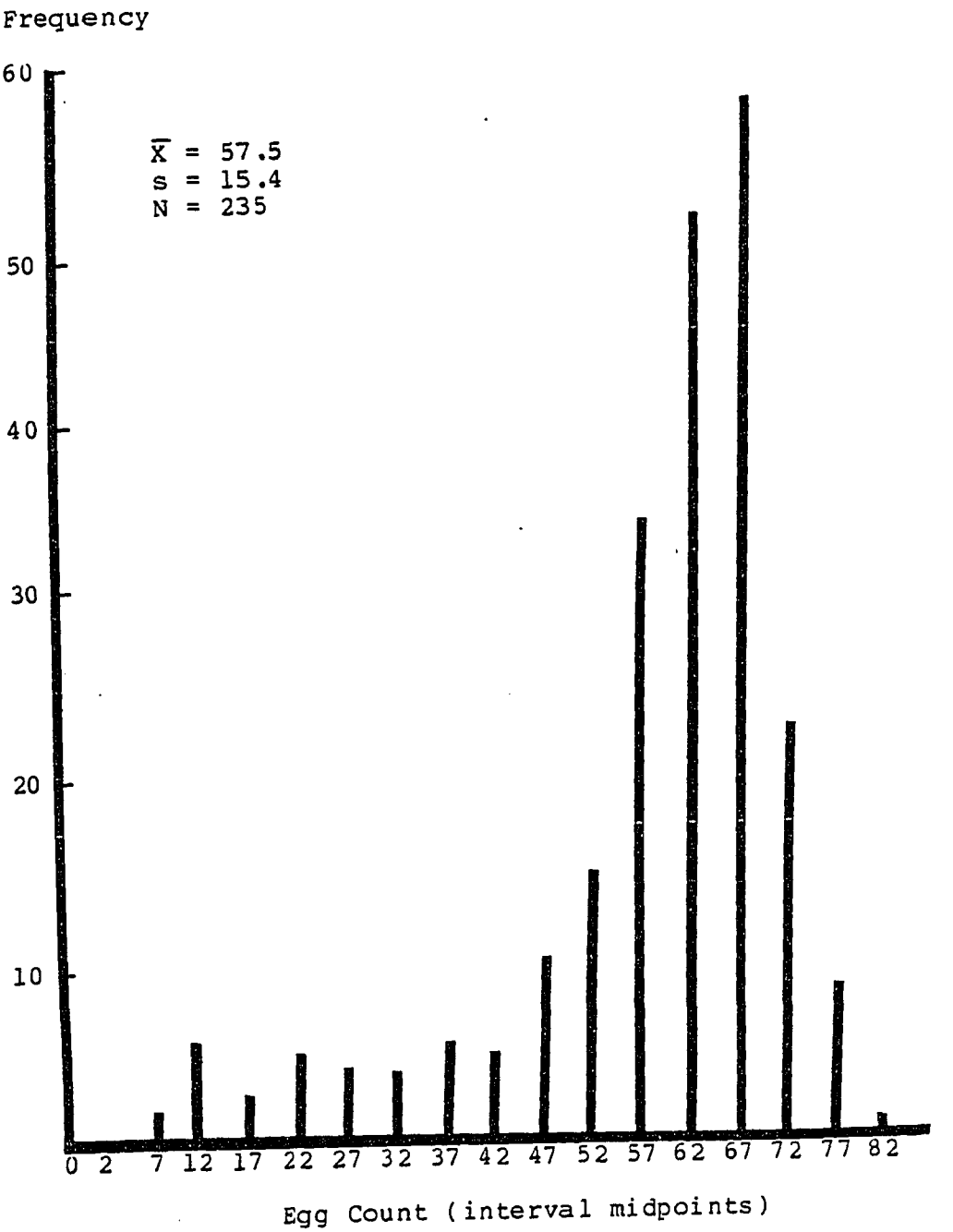


Fig. 2. Frequency of egg counts/mass in multiple choice greenhouse experiment

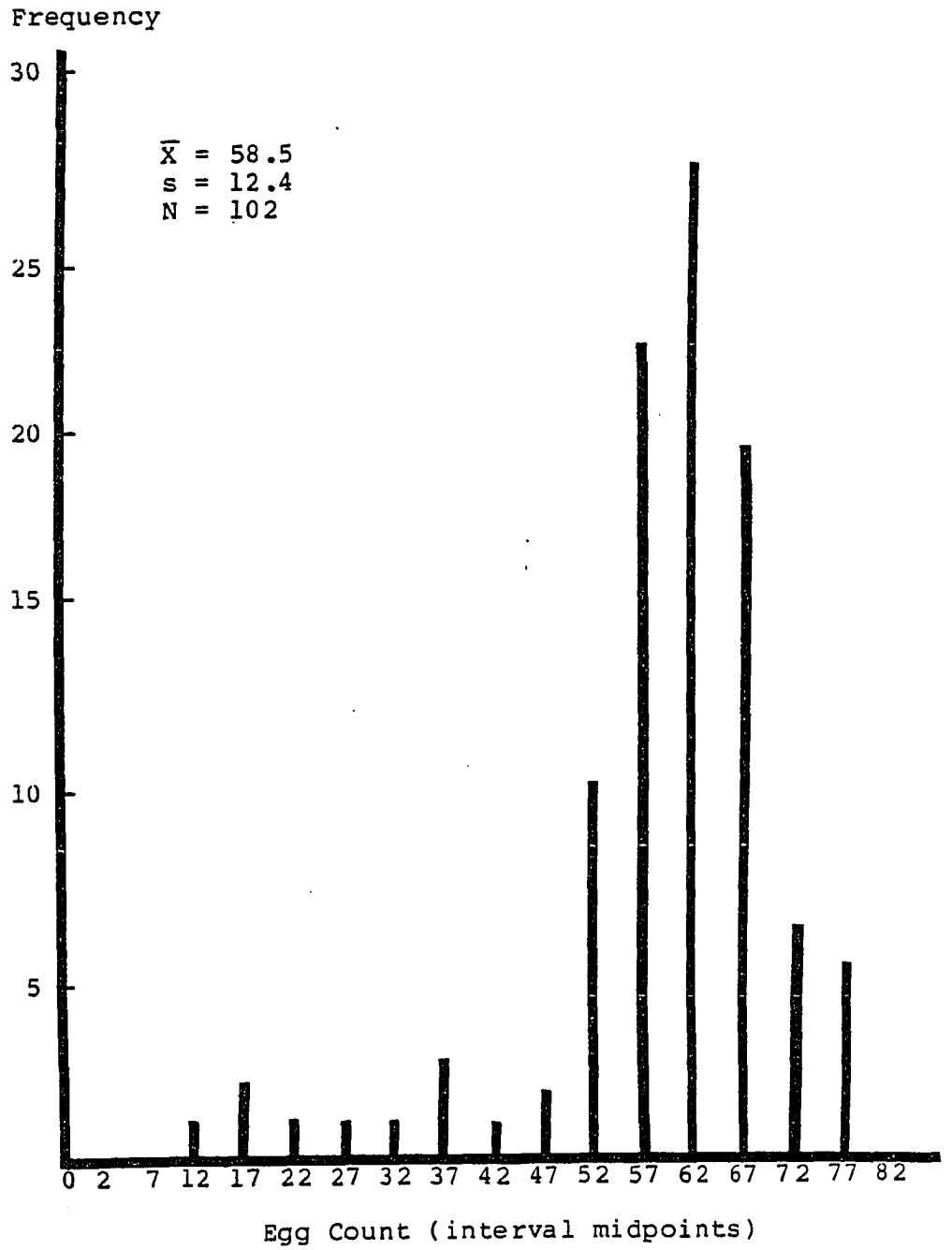


Fig. 3. Frequency of egg counts/mass in no choice greenhouse experiment

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